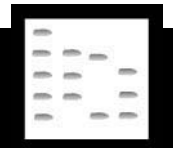
van Orsouw NJ, Li D, van der Vlies P *et al.* (1996) Mutational scanning of large genes by extensive PCR multiplexing and two-dimensional electrophoresis: application to the RB1 gene. *Human Molecular Genetics* 5: 755–761.

van Orsouw NJ, Dhanda RK, Rines DR *et al.* (1998) Rapid design of denaturing gradient-based two-dimensional electrophoretic gene mutational scanning tests. *Nucleic Acids Research* 10: 2398–2406.

Vijg J and van Orsouw NJ (1999) Two-dimensional gene scanning: exploring human genetic variability. *Electrophoresis* 20: 1239–1249.

GEOCHEMICAL ANALYSIS: GAS CHROMATOGRAPHY AND GC-MS



R. P. Philp, University of Oklahoma, Norman, OK, USA

Copyright © 2000 Academic Press

Introduction

Geochemical analysis, and more specifically chromatography, is concerned with samples derived from two different sources: those of relatively recent origin, related to environmental problems; and those of a much greater geological age, related to fossil fuel exploration and exploitation. The chromatographic techniques utilized to analyse and characterize such samples are virtually identical regardless of the age and origin of the sample. The extracts from geochemical samples, whether they are rocks, soils, crude oil spills, contaminated wildlife or spills of refined products, are very complex mixtures of a wide variety of organic compounds. Compounds derived from fossil fuels typically will be complex mixtures of hydrocarbons, and the environmental samples from more recent sediments probably will contain a variety of other compounds such as chlorinated compounds, pesticides or herbicides. In view of the similarities of the techniques used for analysing the samples from these different sources, the majority of examples used in this article to illustrate the techniques will be based on the characterization of fossil fuel samples.

The major goal of any geochemical analysis is to take a sample and, through a variety of fractionations and analytical techniques, reach a point where either the presence or absence of specific target compounds can be determined, or fingerprints for specific classes of compounds can be obtained and used for correlation purposes. Applications related to petroleum exploration might use such fingerprints for oil–source rock or oil–oil correlation studies, whereas in environmental studies one is more concerned with correlating a spilled product with its original source material or trying to evaluate the extent of removal during clean-up procedures.

Geochemical samples are extremely complex mixtures of a wide variety of compound classes. The analytical techniques commonly used to characterize such mixtures involve some form of chromatography, such as gas chromatography (GC), gas chromatography–mass spectrometry (GC-MS), gas chromatography–mass spectrometry/mass spectrometry (GC-MS/MS), and more recently gas chromatography–isotope ratio mass spectrometry (GC-IRMS). Liquid chromatography (LC) and combined liquid chromatography–mass spectrometry (LC-MS) are also used in certain applications, but not to the same extent as GC and GC-MS. In addition to the analytical chromatographic separations, most geochemical analyses require some sort of fractionation into compounds classes prior to the actual analysis. There are certain cases where total sediment extracts or whole crude oils are analysed directly but generally the mixtures are so complex that an initial fractionation(s) is required to simplify the extracts for subsequent analyses. For example gas chromatograms of many crude oils (**Figure 1**) are dominated by *n*-alkanes but, for the most part, compounds that are of much greater geochemical importance are not readily observable in these chromatograms but are hidden in the baseline of the chromatogram. It should be noted that there are also many naphthenic crudes not dominated by *n*-alkanes, e.g. Venezuelan and Russian crudes. Most of these naphthenic crudes are either severely biodegraded or have been generated at relatively low levels of maturity from sulfur-rich kerogens. A fractionation step involving thin-layer chromatography, column chromatography or liquid chromatography, all of which involve partitioning of components between a liquid and solid phase, leads to the separation of

DropBooks

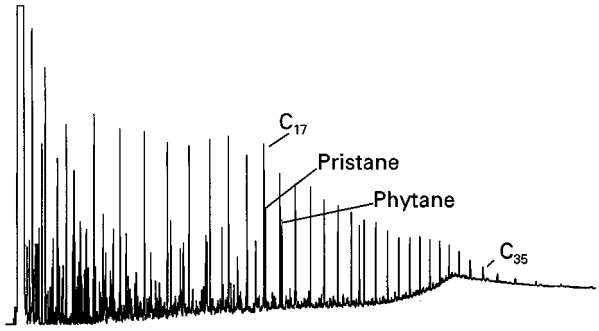


Figure 1 Gas chromatograms of crude oils, rock extracts, or refined petroleum products are typically dominated by *n*-alkanes and isoprenoids. While GC alone does not permit their identification, the fact that the isoprenoids pristane and phytane have very similar elution times to the C_{17} and C_{18} *n*-alkanes, respectively, generally make it relatively easy to identify the other members of the homologous series with a reasonably high degree of confidence.

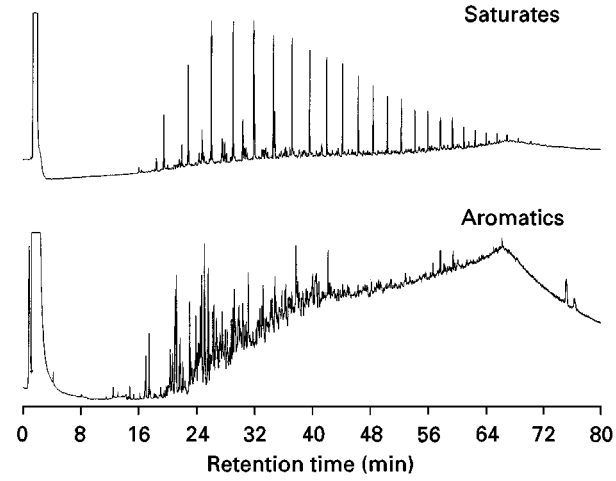


Figure 2 The whole oil chromatogram shown in Figure 1 does not give a true impression of the complexity of the mixture of compounds in a crude oil. While the *n*-alkanes are the dominant components in the chromatogram, a vast array of branched, cyclic, aromatic and polar compounds are also present. This figure shows the chromatograms for a saturate and aromatic fraction separated from a crude oil by thin-layer chromatography.

compounds on the basis of factors such as polarity, shape and size. For hydrocarbon-containing samples, the fractionation step typically involves separation into three fractions – saturate and aromatic hydrocarbons and a polar fraction containing nitrogen, sulfur and oxygen compounds. In general it is the saturate and aromatic fractions that receive most attention in terms of additional analyses. Although the nitrogen, sulfur and oxygen fractions contain many compounds that possess useful information, the complexity of these fractions has precluded their detailed analyses. It is not proposed to go into the experimental details of such chromatographic fractionations since these are very basic techniques and descriptions of specific methods for particular classes of compounds are readily available in the literature.

Fractionation of the crude oil used for Figure 1 into various fractions produces saturate and aromatic fractions as shown in **Figure 2**. It should be noted when comparing Figures 1 and 2 that the result of the fractionation and evaporation of the solvents used in the fractionation process will lead to the loss of some of the more volatile compounds in the C_1–C_{15} range of the saturate and aromatic fractions. GC analyses of the saturate fraction produces a chromatogram dominated by *n*-alkanes, typically in the carbon number range from C_{15} to around C_{40} when the analyses are performed using conventional GC. The isoprenoids pristane (Pr) and phytane (Ph) can also be clearly discerned on these chromatograms. Once again, it should be emphasized that although the *n*-alkanes are the predominant compounds in the chromatograms, there are many more minor compounds in the fraction that generally provide a great deal more information than the *n*-alkanes. These compounds can be further concentrated by such processes as molecular sieving or urea adduction, both of which will separate the *n*-alkanes from the branched and cyclic alkanes as shown in **Figure 3**. At this point fractionation and sieving of the original extract or crude oil will have produced a fraction that is readily

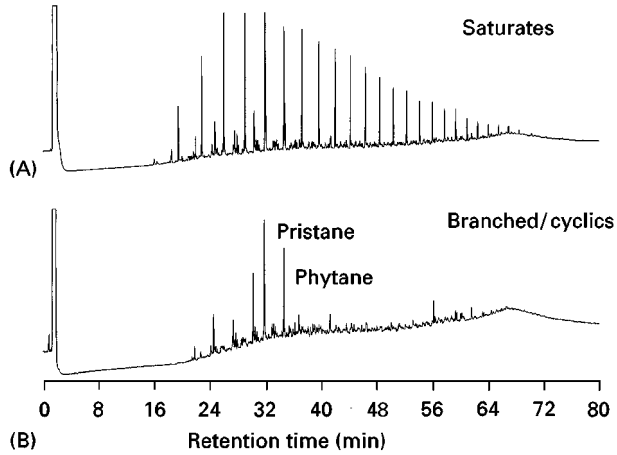


Figure 3 The saturate fraction shown in Figure 2 is again dominated by *n*-alkanes, which tend to mask the presence of a very complex mixture of branched and cyclic compounds also present in this fraction. The *n*-alkanes can be separated from these branched and cyclic compounds by processes such as molecular sieving or urea adduction to produce the branched and cyclic fraction shown in the bottom chromatogram of this figure. The top chromatogram (A), shown for comparison purposes, is the total saturate fraction from which the branched and cyclic compounds were isolated.

amenable to analysis by the techniques mentioned above, such as GC, GC-MS, GC-MS/MS or GC-IRMS. In the following sections a brief description of each technique and typical applications will be given. Again it should be reiterated that for the most part this article uses hydrocarbons for illustration purposes, but the majority of the techniques are equally applicable to the analysis of other samples of geochemical interest such as environmental samples, possibly with some slight modifications in the operating conditions.

Gas Chromatography

As can be seen from the chromatograms used in the Introduction, GC provides a great deal of information on the composition of geochemical samples. With the inclusion of an internal standard, this information can be both quantitative and qualitative in nature. However, it is important to remember that, for the most part, GC does not provide any information on the identification of individual components. In the saturate hydrocarbon fractions, individual compounds such as *n*-alkanes in the chromatograms can be readily recognized and their identification confirmed either by the use of co-injected standards or analysis of the sample by GC-MS as described below. In other cases where it may be necessary to detect certain classes of chlorinated compounds, or organosulfur compounds, additional information on the presence or absence of these compounds can be obtained by using detectors that are specific for these classes of compounds, such as electron capture, flame photometric or Hall detectors. It should also be noted that a number of recent studies have shown that atomic emission detection (AED) is a particularly sensitive and specific method of detection for sulfur-containing compounds. Although the flame photometric detector (FPD) is probably the most widely used detector for sulfur-containing compounds, it has some drawbacks including nonlinear compound-dependent response, and the quenching of sulfur signals by co-eluting hydrocarbons. The AED has a linear response and is compound-independent, permitting easy calibration using any sulfur-containing compounds. This feature is unique because other detectors require the construction of curves using target analytes as standards, which becomes a very time-consuming exercise.

GC has been utilized widely in geochemistry since the 1950s. As column technology and instrumentation have improved, so has the quality of the analytical data. There are many similarities between the gas chromatographs of today and the systems that were developed in the 1950s and 1960s. Detectors and injectors have improved and temperature control of the ovens has improved, but probably the greatest advances have occurred in the field of column technology. Early columns were short, large-diameter packed columns made of stainless steel or copper. Over the years narrow-bore capillary columns were developed, initially made of stainless steel, then glass and more recently fused silica. At the same time as the evolution of capillary columns, the variety of liquid phases for different applications has also greatly improved. The most recent advance, and one that is quite significant for petroleum exploration and production, has been the development of high temperature GC phases. Traditionally most crude oil analyses have characterized hydrocarbons in the C_1–C_{40} range but the advent of high temperature GC (HTGC) significantly changed the way in which we look at hydrocarbon distributions of crude oils. The use of HTGC has demonstrated that many crude oils contain a wide range of hydrocarbons significantly above C_{40}, extending to as far as C_{100} and possibly higher. This in turn has also led to changes in one of the very basic premises of geochemistry – that oils with a high wax content were thought to be derived only from higher plant sources. It is now clear that such oils may also be derived from lacustrine and marine source rocks as a result of analysing a number of samples from such source rocks using HTGC. **Figure 4** provides an excellent illustration of the additional information obtained from the use of HTGC. The upper chromatogram shows the analysis of an ozocerite extract by conventional GC and the bottom chromatogram shows the same sample analysed by HTGC. Clearly the distribution of hydrocarbons is quite different in the lower chromatogram. The significance of this is related to the fact that the greater the concentration of the higher molecular weight alkanes, the greater the production problems associated with oils that contain such compounds. In other words, if the oils were only characterized by conventional GC, high molecular weight hydrocarbons would remain undetected. Once a production programme was initiated it would not be long before the wellhead facilities and pipelines would become blocked with paraffin deposits, which require costly measures to remove. While HTGC analyses do not eliminate the problem, production engineers would be aware of the potential for such a problem and steps could be introduced to minimize its occurrence.

With the availability of HTGC an increasing number of samples have been analysed using this approach and steps have been taken to develop methods that will quantitatively separate high molecular weight alkanes from the asphaltene fraction. Analyses

DropBooks

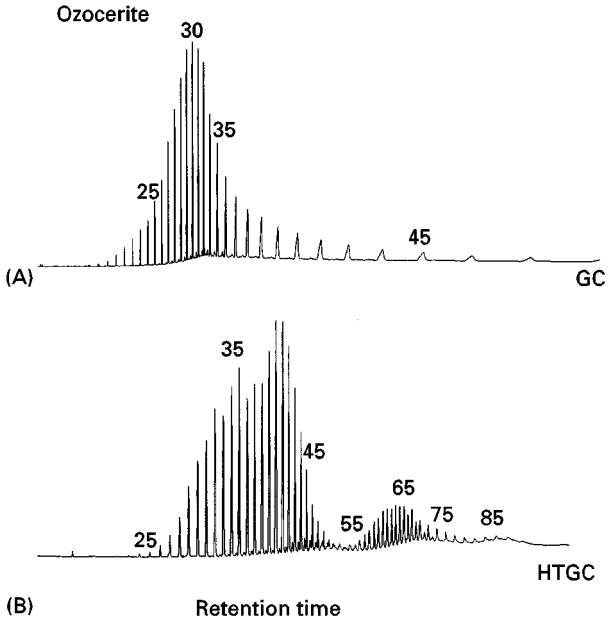


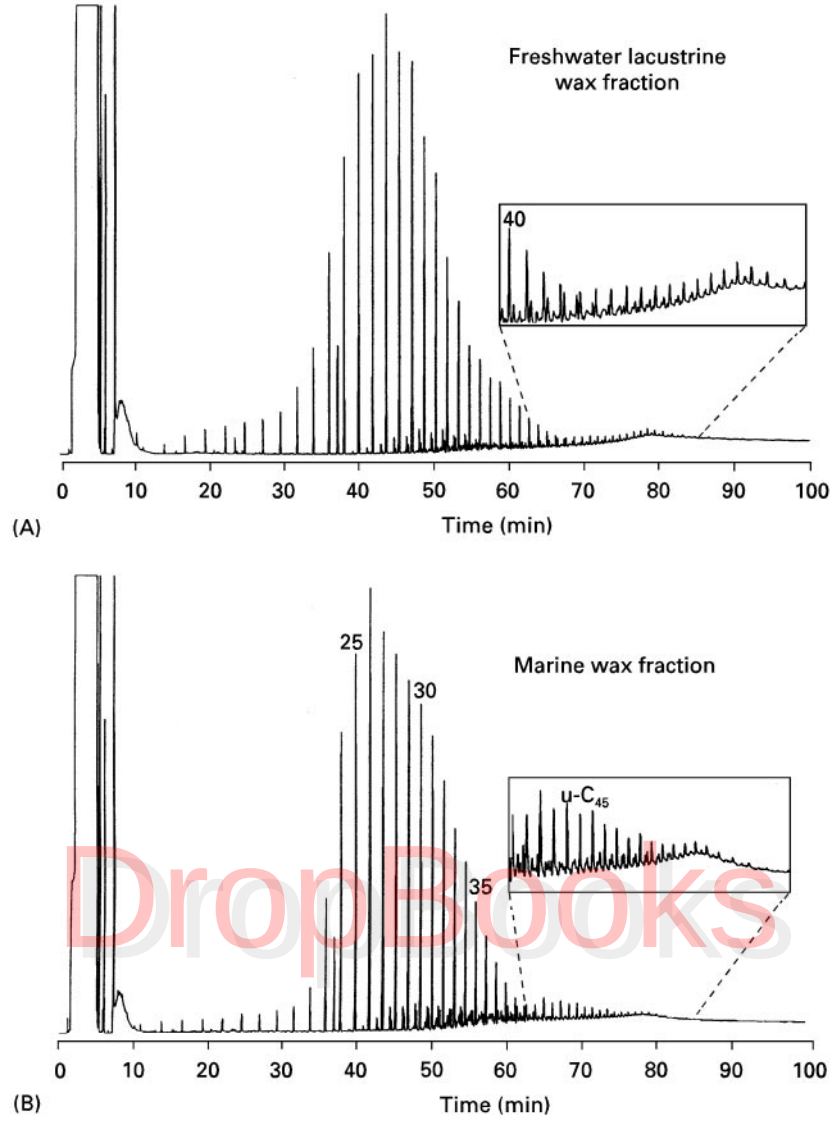
Figure 4 One of the most significant recent developments within GC has been the development of high temperature phases for the columns. Before this development it was generally only possible to analyse compounds with up to approximately 40 carbon atoms. The newer HTGC columns permit samples containing up to approximately 120 carbon atoms to be analysed. This figure illustrates the comparison between the analyses of the hydrocarbons in the fossil bitumen ozocerite by (A) conventional GC and (B) HTGC. The difference in distributions is very clear and also demonstrates how the composition of a fraction obtained by conventional GC may not necessarily reflect the true composition of the sample being investigated.

of a wide range of such samples have shown that, in addition to the *n*-alkanes, there is also a wide range of additional compounds in the higher molecular weight fraction including branched alkanes and alkylcyclohexanes. The distribution patterns for these compounds has provided an additional powerful tool for determination of the type of environment from which a sample has been generated. For example **Figure 5**A and **B** show the distributions of high molecular weight fractions from oils whose source materials were known to be deposited in lacustrine and marine depositional environments, respectively. Note the difference in the distributions of these monocyclic hydrocarbons, which are characteristic of the different environments. At present relatively little information is available concerning the origin of these compounds, although their widespread distribution suggests that they are probably related to an algal/phytoplanktonic source, possibly with additional contributions from higher plant waxes. It is known that many marine organisms contain abundant quantities of higher molecular weight esters, alcohols and fatty acids. Relatively simple transformations could readily convert these compounds to the corresponding hydrocarbon and could easily represent a viable source for such hydrocarbons.

Another area of geochemistry that has become particularly important in the past few years is reservoir geochemistry, and GC has played an extremely important role in its development. Oil and gas reservoirs are very complex geological features with many compartments. A knowledge of the relative position of these compartments is extremely important for reservoir management, determination of where additional production wells should be drilled, and evaluating how a specific reservoir may have been filled. There are a number of ways in which the reservoir compartments may be delineated but one particularly interesting, innovative and relatively cheap method involves the utilization of high resolution GC. As indicated above, crude oils are very complex mixtures of hydrocarbons, but when the chromatograms are expanded the complexity of the mixtures becomes far more apparent and the presence of a large number of minor components is clearly visible. Reservoir geochemistry utilizes these minor components to assist in the delineation of the reservoir compartments. In brief it is first necessary to determine whether all of the oils in the reservoir are derived from the same source materials. Once this has been established, all of the oils need to be analysed by high resolution GC, the early eluting region of the chromatogram expanded and a number of pairs of minor peaks selected as shown in **Figure 6**. Ratios based on pairs of selected peaks are measured and subsequently plotted on a star or polar diagram. This process is then repeated for all the oils to be examined from the reservoir. It is important to ensure that the same pairs of peaks are selected for each oil, even if the identity of these peaks in unknown. Since the differences between the pairs of peaks in individual samples are often quite small it is extremely important to ensure that the GC analyses are highly reproducible for this particular application. However, once all of the oils have been analysed and the data plotted on the star diagram, it will be found that oils that are in the same compartment or in communication will appear virtually on top of each other, whereas those oils in different compartments will be slightly separated (**Figure 7**). These small difference may result from slight differences in oil–rock interactions, slight maturity differences or generation from slightly different sources.

Gas Chromatography–Mass Spectrometry

While GC can provide a great deal of information that is of interest and useful from a geochemical



DropBooks

Figure 5 The availability of HTGC has led to the discovery of numerous new series of compounds present in oils and source rocks above C_{40}. Several of these series, and in particular a series of alkylcyclohexanes, have been shown to be useful in discriminating between oils derived from source materials deposited in lacustrine (A) versus marine settings (B). More subtle variations in these distributions allow the salinity levels of the depositional environments to be distinguished.

perspective, it should also be noted that in most cases GC only provides information on the distribution of the major components in the sample, and for the most part these are generally dominated by the *n*-alkanes. The more useful compounds are the more complex molecules, or biomarkers, which are typically present in relatively low concentrations and which require the use of GC-MS and more specifically single ion or multiple ion detection (MID) in order to determine their distributions. While there are many classes of biomarkers that are commonly used for correlation and other purposes, compounds such as the steranes and terpanes will typically provide the greatest amounts of useful information for both an environmental and exploration context.

To illustrate the utility of the biomaker fingerprints, the gas chromatograms of three oils are shown in **Figure 8**. From the gas chromatograms alone it is virtually impossible to determine what relationship, if any, exists between these samples. In other words, are they from the same source rock or can they be correlated with each other? The effects of biodegradation are clearly evident in sample B since all of the *n*-alkanes have been removed, making it appear even more significantly different from the other two samples. Detailed analyses of the same samples by

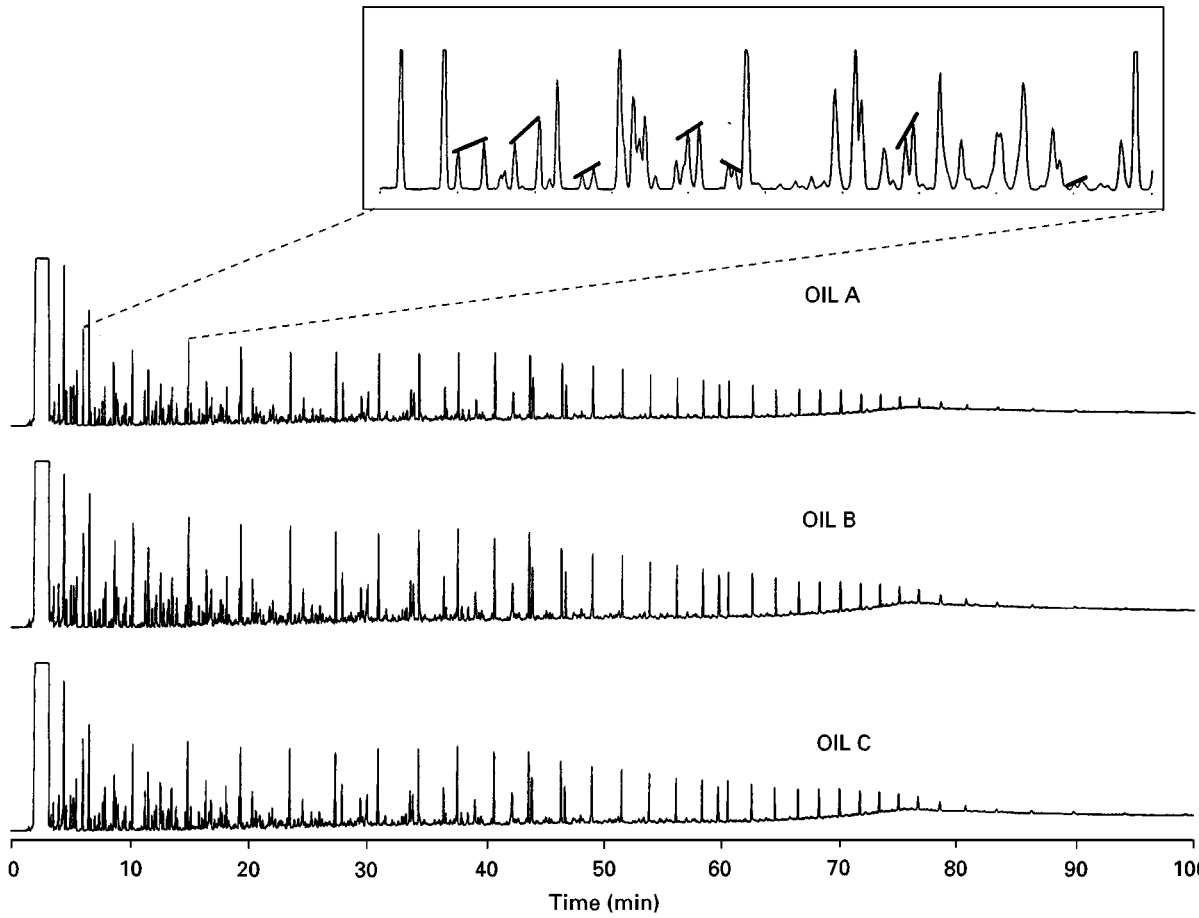


Figure 6 Reservoir geochemistry has provided an important means of determining continuity and communication within reservoir compartments. Once it has been established that the oil in a reservoir is from a common source, high resolution gas chromatograms are obtained for individual samples and ratios of various pairs of peaks are determined, as shown in the figure. The identity of the components does not have to be known; the important point is that the same pairs of peaks are used for all the samples examined in any particular study. Although the early studies typically used peaks in the early part of the chromatogram, it has been shown that the minor components in the higher regions of the chromatogram can also be used for the same purpose.

GC-MS and MID using the characteristic ions for the sterane and terpane biomarkers at m/z 217 and 191, respectively, produces the additional data shown in **Figure 9**. On the basis of the chromatograms shown in Figure 9, samples B and C are in all probability related to each other. It is not necessary to identify each component, rather one should think of the mass chromatograms as fingerprints. If two samples are derived from the same source, then their fingerprints should be the same, or at least very similar; samples from different sources will be different from each other. Hence when the fingerprint for sample A is compared with those for B and C in Figure 9, there are a number of significant differences between these samples that permit one to conclude that A is from a different source than B or C. The biomarker fingerprints obtained in this way are very specific for a variety of applications, in addition to this type of correlation. The presence of individual compounds, for example oleanane and gammacerane, can provide information on the presence of specific types of source materials or the nature of the depositional environment.

To illustrate this type of application, **Figure 10** shows the m/z 191 and 217 mass chromatograms of an oil that is derived from source material dominated by higher plant or terrestrial source material. This evidence is contained in the fact that the predominant component in the m/z 191 mass chromatogram is the terpane called 18α(H)-oleanane. It has been established that this compound has its precursor in higher plant material and hence the presence of this compound in an oil will indicate that the sample is derived from such material. In support of such evidence is the

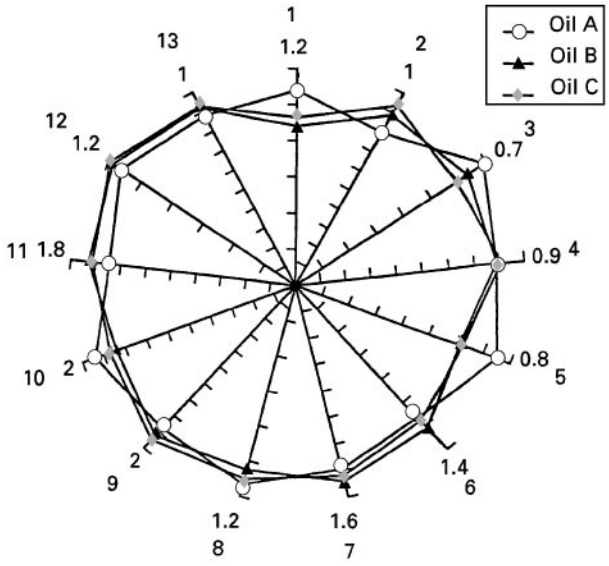


Figure 7 The ratios of the pairs of peaks measured in Figure 6 are plotted on a star or polar diagram. If two oils are in the same compartment, or in communication with each other, then on such a star plot the two oils will have identical plots (i.e. B and C). If they are not in communication with each other, then their plots will show some subtle differences (i.e. oil A).

fact that the sterane chromatogram is dominated by the C_{29} steranes. For oils of this nature it has been clearly established that the C_{29} steranes are also associated with higher plant source materials. In this manner, pieces of evidence can be put together that in many cases will provide a very clear indication as to the origin of the material being analysed.

In the second example, shown in **Figure 11**, the presence of another very specific compound, gammacerane, can also be very clearly seen in the *m/z* 191 chromatogram. This compound is a very specific indicator of depositional environments of enhanced salinity. Recent attempts have been made to relate the presence of certain compounds, for example, dinosterane to the age of the source rock from which the sample was generated. Specific ratios of different sterane isomers or terpane isomers are also used extensively for determining the relative maturity of oils or source rocks.

The sterane and methylsterane distributions in crude oils are far more complex than the terpanes and no matter how good the GC resolution, it is impossible to obtain complete separation of all co-eluting isomers, epimers and homologues (**Figure 12**). In order to optimize this separation it is necessary to utilize GC combined with tandem mass spectrometry, or MS/MS, which provides an additional degree of separation based on the utilization of the MS/MS capability.

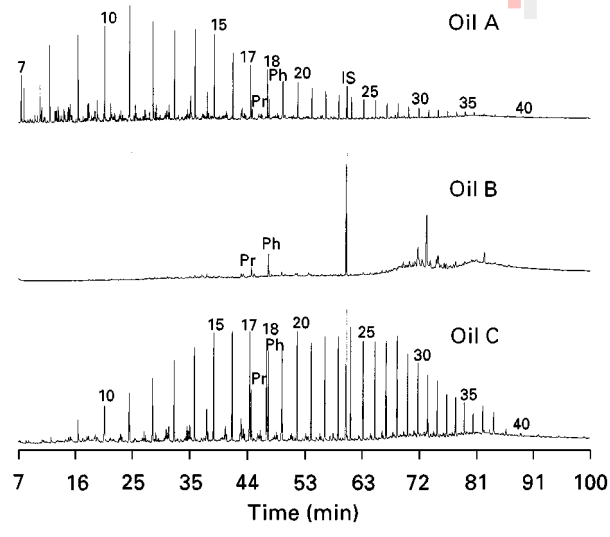


Figure 8 Gas chromatograms of three different oils provides limited information concerning the relationship between the samples. In this figure oil B clearly appears to be quite different from oils A and C, but all that can really be said is that oil B has been heavily biodegraded and the *n*-alkanes have been removed. It is impossible to say what, if any, similarities there may have been between the alkane distribution for this sample and the other two samples.

Gas Chromatography–Mass Spectrometry/Mass Spectrometry

To demonstrate the utility of the GC-MS/MS approach to the characterization and determination of biomarkers in geochemical samples, the resolution of a complex mixture of sterane isomers and homologues will be described. While this example utilizes the steranes, it should be borne in mind that the same approach can be used to resolve any very complex mixture of organic compounds from geochemical samples.

The mixture of steranes commonly analysed by MS/MS is in the C_{27}–C_{30} carbon number range and each homologue has a molecular mass at *m/z* 372, 386, 400 and 414, respectively. For each of the steranes the parent ions will undergo a collision-activated decomposition to produce a daughter ion at *m/z* 217. Hence a series of MS/MS parent–daughter experiments are performed utilizing these parent–daughter transitions in combination with the GC separation. The GC-MS analysis and single ion monitoring of *m/z* 217 produces the mass chromatogram shown in Figure 12 but with the GC-MS/MS analyses, the results shown in **Figure 13** are obtained. It can be seen in Figure 13 that by using the C_{27} parent–daughter ion pair at *m/z* 372/217, respectively, the result of analysing the sample by MS/MS is to

DropBooks

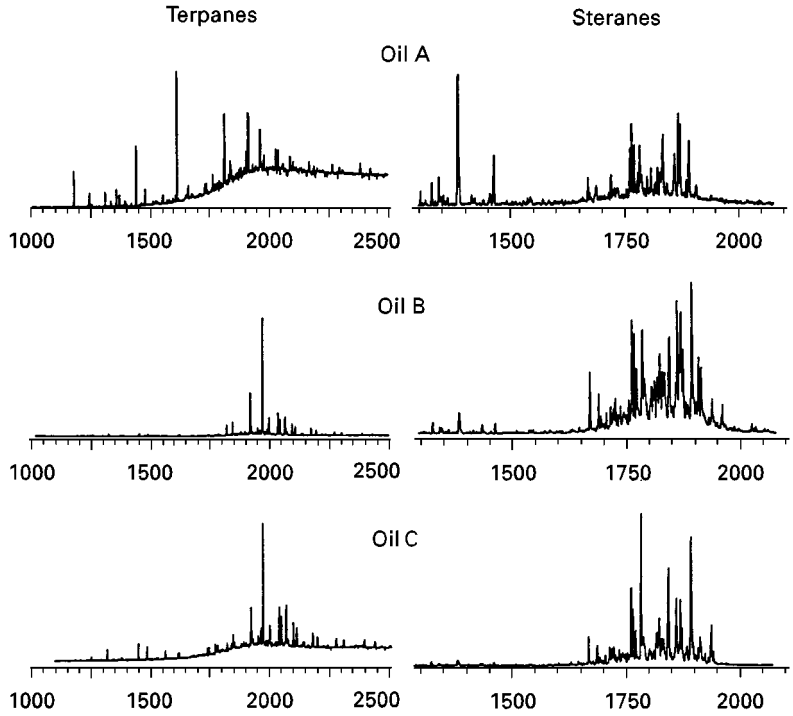


Figure 9 The terpane and sterane distributions for the same three oils as used in Figure 8 provide a more specific indication of the relationship between different samples. It is not necessary to know the identification of all the individual peaks but rather to think of the total chromatogram as a fingerprint. In this case it can be seen that oil A has quite a different set of fingerprints than oils B and C, suggesting it is not related to these other two samples. While the fingerprints of oils B and C may show some differences, these are actually due to small maturity differences in the samples.

totally resolve the C_{27} components from the rest of the complex mixture.

Similar results would be obtained if the parent–daughter pairs for the other members of the series were also illustrated. A similar approach could be applied to the methylsterane mixture using the parent ions and the daughter ion at mass 231 and a similar simplification of the mixture would be obtained. In this particular application the MS/MS serves to introduce an additional element of separation following the initial separation by GC.

Pyrolysis–Gas Chromatography–Mass Spectrometry

While a large proportion of the geochemical samples analysed are soluble in organic solvents and readily amenable to direct analysis by GC or GC-MS, there is another aspect to geochemical samples that is often overlooked. Samples of geochemical interest such as soils, source rocks or coals also have a significant insoluble organic component such as the humic fraction of soils or the kerogen fraction of a source rock. Characterization of these insoluble fractions requries some type of degradation step prior to analysis. At present for geochemical purposes this degradation step typically consists of some type of pyrolysis reaction with the pyrolyser interfaced to the gas chromatograph or GC-MS system. There are also reports of the use of various NMR techniques to characterize this insoluble fraction, although this is a little less specific than the pyrolysis approach.

An example of the pyrolysis of the insoluble fraction of an organic-rich source rock in shown in **Figure 14**. This was produced by pyrolysing the sample at a temperature of 600°C for a short period of time and allowing the pyrolysis products to be transferred directly to the GC column. (There are of course a wide variety of pyrolysis conditions that could be used, but those cited here give a general idea of the typical conditions used.) The products of a sample pyrolysed in this manner produce a chromatogram dominated by alkane/alkene doublets plus a wide variety of minor components. From these distributions it is often possible to gain information about the nature of the source materials originally responsible for the formation of the kerogen plus the type of products it will subsequently produce if buried to

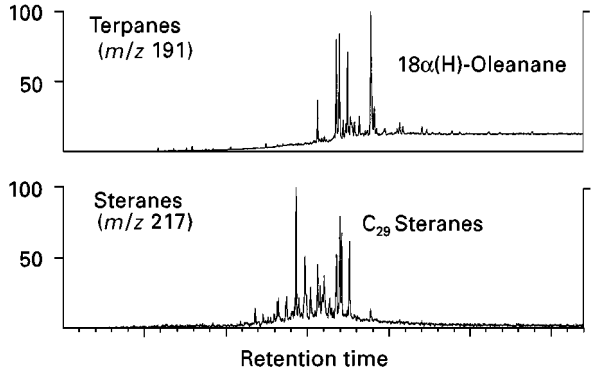


Figure 10 GC-MS analysis of crude oils reveals complex fingerprints of biomarkers. In many cases these compounds may be very specific indicators of particular types of source materials responsible for sourcing the oil. In this example the predominant component in the terpane chromatogram is 18α(H)-oleanane. Not only is this compound very specific in terms of being derived from higher plant source materials, but it is also more specifically related to the flowering plants or angiosperms that have only evolved since the Late Cretaceous–Early Tertiary periods. The presence of this compound can therefore be used to constrain the age of the source rock from which the oil was generated.

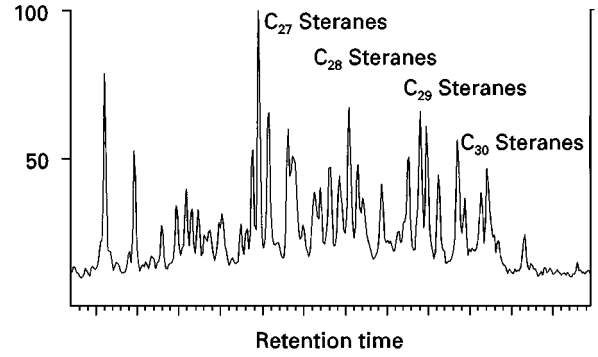


Figure 12 Sterane distributions in crude oils as typically determined by single ion monitoring of *m*/*z* 217 reflect the complex nature of the mixture of these components in a crude oil. It is virtually impossible to separate all of the co-eluting isomers, epimers and homologues by GC-MS, no matter how good the chromatography.

appropriate depths and subjected to thermal degradation.

Another useful application is the pyrolysis of asphaltenes, particularly those isolated from biodegraded crude oil samples. It is often difficult to determine the origin of a biodegraded oil sample. However, if there are a number of possible nondegraded samples with which it can be compared, then the asphaltenes can be isolated from all the samples by pentane precipitation and pyrolysed. In this way it will be observed that the *n*-alkane/alkene fingerprints generated from the degraded and nondegraded samples will be virtually identical if the samples are derived from the same source, but quite different if the samples are unrelated.

PropBooks

Gas Chromatography–High Resolution Mass Spectrometry

The majority of geochemical analyses reported in the literature are concerned with the detection and identification of hydrocarbons. However, many

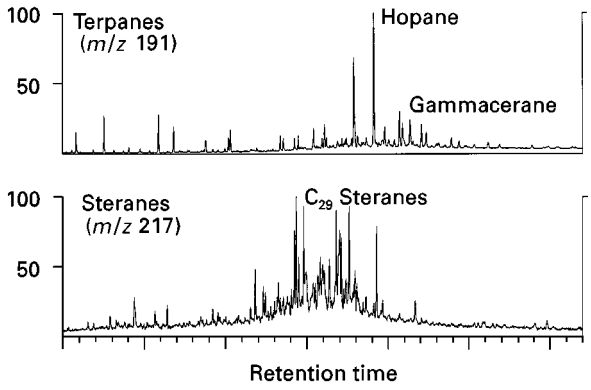


Figure 11 Gammacerane is another very specific biomarker that can be readily observed in the *m*/*z* 191 chromatogram. The relative concentration of this compound varies with the salinity of the original depositional environment. Hence samples deposited in a very saline lacustrine setting will generally have high gammacerane contents whereas those from freshwater lacustrine environments are generally depleted in gammacerane.

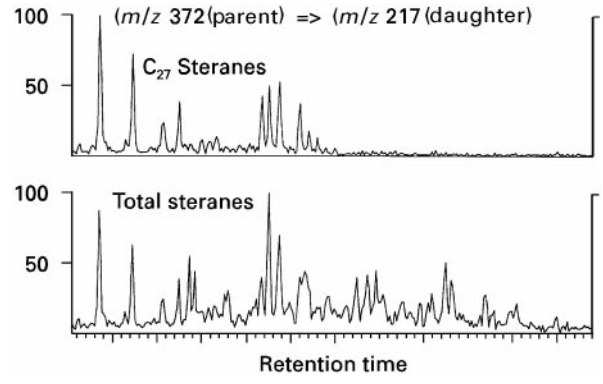


Figure 13 To simplify the sterane fingerprint to some degree GC-MS/MS becomes an important tool. In this diagram the same sterane mixture used for Figure 12 has been analysed in the GC-MS/MS mode. By filtering out the parent–daughter ions for the C_{27} steranes, the top chromatogram shows only the C_{27} compounds. Distributions for the C_{28}–C_{30} steranes could also be obtained using the appropriate parent–daughter ions.

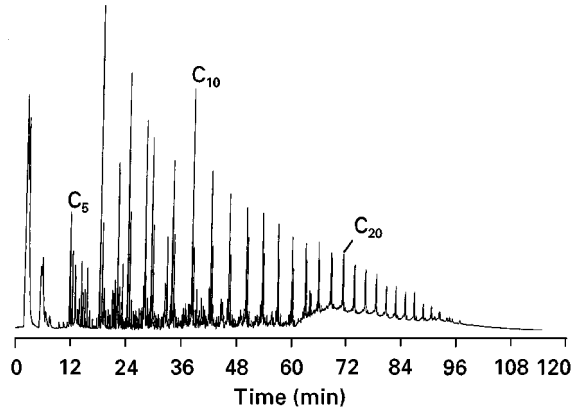


Figure 14 One of the most useful ways for characterizing the insoluble organic matter in a source rock, coal, shale or soil sample is by pyrolysis-GC. This figure illustrates the results obtained from pyrolysis-GC of Messel shale and shows that the major components obtained in the approach are a series of alkane/alkene doublets. Variations in these distributions can be used to characterize the organic matter in terms of whether it is algal or terrestrial as well as provide information on the relative maturity of the samples.

geochemical mixtures contain complex mixtures of compounds in which heteroatoms are mixed with the hydrocarbons in varying amounts. In certain applications knowledge of these components, particularly sulfur-containing compounds, may be extremely important. There are two approaches by which such distributions may be obtained. The first is by the use of element-selective GC detectors, such as the FPD, Hall detector, or one of the more recent types of AED that are selective for sulfur-containing compounds at the exclusion of non-sulfur-containing compounds. The second method combines GC with high resolution MS. Although this approach is not used routinely, it is a very powerful and specific technique for this type of application, as discussed by Tibbetts and Large in 1988. While the use of low resolution GC-MS and ancillary techniques such as single ion monitoring and multiple ion detection have been discussed elsewhere, it needs to be recognized that utilization of nominal masses in MID may lead to ambiguous results. As demonstrated by Tibbets and Large, while the ion at nominal mass 184 may be used for the determination of dibenzothiophenes (DBT), it is also the nominal mass for the C_4-substituted naphthalenes, leading to possible misinterpretation of the resulting chromatograms. However, use of the accurate mass at m/z 184.0347 permits detection of only the DBT and no substituted naphthalenes. Several examples have been given by Tibbetts and Large on the use of this approach for the correlation of crude oils or to distinguish oils from different sources or reservoirs. Used in conjunction with the conventional detection of biomarkers, this method provides a very powerful and additional tool for geochemical analyses.

Gas Chromatography–Isotope Ratio Mass Spectrometry

Carbon naturally exists as a mixture of its two stable isotopes, ^{12}C and ^{13}C, in an approximate $^{12}C/^{13}C$ ratio of 99 : 1. The carbon isotopic composition of living organic matter in part depends on the species but is also determined by a number of environmental properties. For example, atmospheric carbon dioxide is assimilated by living plants during photosynthesis and the nature of the plants and whether they assimilate CO_2 via a C3 or C4 photosynthetic cycle will determine the extent of preferential assimilation of the lighter ^{12}C isotope. C3 plants are typically associated with warmer and more arid climates and in general have isotopic values in the -10 to $-18‰$ range. C4 plants are more typically associated with colder climates and have lighter isotopic values in the -22 to $-30‰$ range. To determine the ^{13}C composition, the sample is combusted to convert all of the carbon to CO_2, which is analysed in a stable isotope ratio mass spectrometer and compared with the isotopic composition of a standard material (Pee Dee Belimnite, PDB), whose isotopic composition has been assigned a value of 0.

GC-IRMS and Isotopic Composition of Individual Components

GC-IRMS permits acquisition of $\delta^{13}C$ values for individual components in complex mixtures. The important part of the system is the interface between the GC and the isotope ratio mass spectrometer. This consists of a reactor tube, generally a narrow-bore ceramic tube, containing a bundle of wires, typically copper, nickel or platinum where complete combustion to CO_2 must occur. After combustion the water and CO_2 pass through a membrane separator to remove the water before the CO_2 enters the mass spectrometer, where the isotopic composition of the gas is determined relative to the standard. The isotopic values of individual components can be interpreted to obtain information on the diagenetic history of an individual component and the nature of the microbial community during deposition.

The isotopic composition of individual compounds is also of importance from an environmental viewpoint. For example, analysis of a whole oil, or the saturate fraction of a whole oil, allows the ready determination of $\delta^{13}C$ values of the *n*-alkanes and the

major isoprenoids, pristane and phytane. These values can be used for correlation purposes, to distinguish oils from different sources, to correlate oil spills with their suspected sources, or to determine the source of hydrocarbons that have contaminated wildlife. Examples of this approach are shown in **Figure 15**. Gas chromatograms of oil extracted from the feathers of birds that had been exposed to a crude oil spill and a suspected source are compared with each other and show certain differences, particularly at the lighter end of the chromatograms. Such differences could lead to a dispute as to whether or not this oil was actually the one that was responsible for contaminating the birds. The lower part of the figure shows the carbon isotope data for individual compounds in the two samples. It can be seen that, despite the loss of some of the light ends through evaporation, a good relationship between the two samples can be established. These data could also be used in support of the biomarker and other properties normally used to establish relationships between samples thought to be related. In an additional example, **Figure 16** shows the results from the GC-IRMS analyses of 20 oils from a region in SE Asia. It can be seen that on the basis of these analyses two distinct families of oils are present in the region. One family has isotopic values of around $-20‰$ for each compound whereas the other family has values around $-28‰$. This information can be used in conjunction with the biomarker data to determine the significance of these differences.

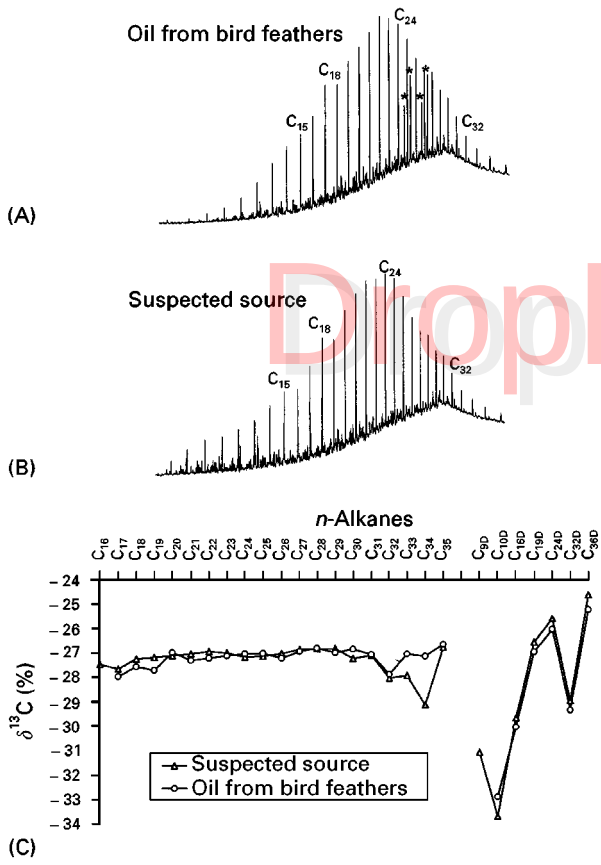


Figure 15 (A) Chromatogram of an extract from bird feathers contaminated by a crude oil; (B) shows the chromatogram for the suspected source of the contamination. The two chromatograms show some significant differences, particularly in the early part of the chromatogram, mainly as a result of weathering. However, in (C) we see that there is a strong relationship between the isotopic compositions for each individual *n*-alkane in the two samples. This isotopic information can be used in conjunction with the biomarker data and other parameters to establish a relationship between the two samples.

While these are just two examples, we have also shown that GC-IRMS can be used in an environmental context to correlate weathered and unweathered oils and refined products and their weathered counterparts. If there are small amounts of the *n*-alkanes remaining it should be possible to obtain their isotopic composition and subsequently use these values to make the correlation. Alternatively if the samples have been so extensively biodegraded that all of the *n*-alkanes have been removed, it is possible to isolate the asphaltenes, pyrolyse them and analyse the pyrolysates by GC-IRMS. Correlations can then be made using these data. This is a particularly valuable approach for the correlation of refined products that only contain lower carbon number compounds and none of the more reliable biomarker compounds that are typically used for correlation purposes.

DropBooks

Summary

This article has attempted to illustrate the importance of GC to geochemical analyses. Geochemical samples from all sources, whether recent or ancient, oils or synthetic chemicals, refined or crude, are incredibly complex mixtures of organic compounds in most cases. To try and analyse such samples, whether simply for correlation purposes or to detect and identify unknown compounds, almost inevitably requires some level of chromatography to facilitate the analytical process. The most common forms of chromatography generally involve some form of liquid chromatography in the initial steps to simplify the mixture into compound classes, followed by GC to separate and resolve as many compounds as possible in the resulting fractions. Chromatography alone simply separates the components, hence it is very common in most geochemical analyses for the chromatographic step to be combined with an identification technique such as MS. The results of such

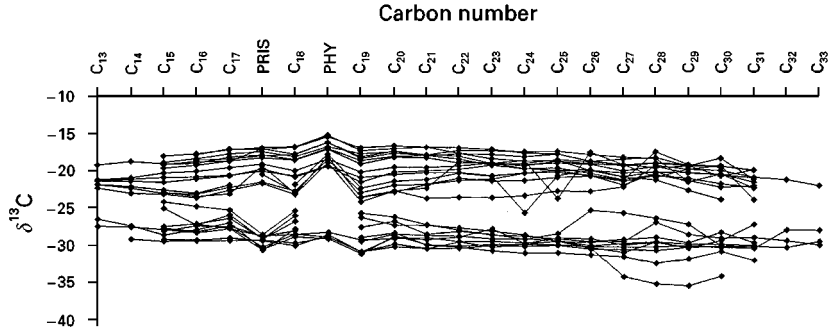


Figure 16 Analysis of 20 oils by GC-IRMS provides isotopic data for each major compound present in the oils. The oils plotted on this chart can be divided into isotopically heavy and isotopically light groups. Supporting evidence for these groupings can be obtained from the GC-MS data.

analyses generally provide the information necessary to determine the origin of a particular sample and, in the context or crude oil exploration, relate it to possible source rocks and such information as age, maturity, and migration pathways. For environmental samples the information obtained is generally for the purpose of determining the source of a spill and hence a great deal of use is made of these distributions in terms of their fingerprinting capability. As chromatographic and spectroscopic techniques continue to improve, clearly the degree of separation achievable for these complex mixtures will also greatly improve, but mixtures of even greater complexities will always be available to provide that next level of challenge.

See also: **II/Chromatography: Gas:** Column Technology; Detectors: General (Flame Ionization Detectors and Thermal Conductivity Detectors); Detectors: Mass Spectrometry; Detectors: Selective; Historical Development; Pyrolysis Gas Chromatography; Theory of Gas Chromatography.

Further Reading

Baskin DK, Hwang RJ and Purdy RK (1995) Prediction of gas, oil, and water intervals in Niger Delta reservoirs using gas chromatography. *American Association of Petroleum Geologists Bulletin* 79: 337–350.

Brooks J and Fleet AJ (eds) (1987) *Marine Petroleum Source Rocks*, Geological Society Special Publication, vol. 26, 444 pp. Oxford: Blackwell Scientific Publications.

Carlson RMK, Teerman SC, Moldowan JM, Jacobson SR, Chan EI, Dorrough KS, Seetoo WC and Mertani B (1993) High temperature gas chromatography of high wax oils. In: *Proceedings of the 20th Annual Convention of the Indonesian Petroleum Association*, Jakarta, pp. 483–507.

Connan J (1984) Biodegradation of crude oils in reservoirs. In: Brooks J and Welte DH (eds) *Advances in Petroleum Geochemistry*, vol. 1, pp. 299–335. London: Academic Press.

England WA (1989) The organic geochemistry of petroleum reservoirs. *Organic Geochemistry* 16(1–3): 415–425.

Hayes JM, Freeman KH, Popp BN and Hoham CH (1990) Compound-specific isotopic analyses: a novel tool for reconstruction of ancient biogeochemical processes. *Organic Geochemistry* 16 (1–3): 1115–1128.

Johnson D, Quimby BD and Sullivan JJ (1995) An atomic emission detector for gas chromatography. *American Laboratory*, pp. 13–20.

Killops SD and Readman JW (1985) HPLC fractionation and GC-MS determination of aromatic hydrocarbons from oils and sediments. *Organic Geochemistry* 8: 247–257.

Levy JM (1994) Fossil fuel applications of SFC and SFE: a review. *Journal of High Resolution Chromatography* 17: 212–216.

Peters KE and Moldowan JM (1992) *The Biomarker Guide: Interpreting Molecular Fossils in Petroleum and Ancient Sediments*. Englewood Cliffs, NJ: Prentice Hall.

Philp RP and Oung J-N (1992) Biomarkers, occurrence, utility and detection. In: Voress L (ed.) *Instrumentation in Analytical Chemistry*, 1988–1991, pp. 368–376. Washington, DC: American Chemical Society.

Del Rio JC and Philp RP (1992) High molecular weight hydrocarbons: a new frontier in organic geochemistry. *Trends in Analytical Chemistry* 11 (15): 187–193.

Tibbetts PJC and Large R (1988) Improvements in oil fingerprinting: GC/HRMS of sulphur heterocycles. In: Crump GB (ed.) *Petroanalysis 87*, pp. 45–57. London: John Wiley.